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by

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16. ABSTRACT
Since interaction with deoxyribonucleic acid (DNA) defines the genotoxic end points detected, simple in vitro tests can be used to monitor or screen most chemicals or mixtures of chemicals for specific genotoxic activity. Bacterial tests to screen classes of chemicals for gene mutations can provide a screen for many potential mutagens, carcinogens, and teratogens. Since the biochemical mechanisms for all mutagens, carcinogens, and teratogens are not the same, however, bacterial tests cannot be expected to detect all genotoxicants. Only by clearly understanding a test's usages and limits, can one most effectively use the information provided by that test. For example, mutagenicity tests using bacteria have been available for approximately 30 years. In 1951, Demarec et al. used an Escherichia coli (E. coli) reverse mutation system to test 31 chemicals. Combining many improvements, Ames et al. (1975) published a detailed protocol that incorporated the use of a mammalian metabolizing system, and established the Ames test as a reliable screening system for mutagenicity and potential carcinogenicity. Many have examined, discussed, and suggested modifications and additions to the Ames 1975 protocol (Arimoto et al., 1981; Grafe et al., 1975; Belser et al., 1981; Salmeen and Durstin, 1981; Griem, 1980; Polley and Raineri, 1980; de Serres and Shelby, 1979; Cheli et al., 1980; Thompson and Melampy, 1981; Chen et al., 1975; Shahin and von Borstel, 1978; Matsumoto et al., 1977; Anderson and McGregor, 1980; Booth et al., 1980; and Dunkel, 1979). No other protocol has gained as much general and standard acceptance. A discussion of the Ames test procedure and its special uses, limitations, and controversial aspects is the aim of this chapter.

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A PRIMER ON THE AMES *SALMONELLA TYPHIMURIUM* PLATE INCORPORATION ASSAY FOR MUTAGENICITY

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INTRODUCTION

Many of the benefits of any modern, developing society are accompanied by certain hazards. Most energy sources such as electrical power plants, for example, use either solid or liquid organic fuels and emit a variety of by-products into the air as respirable particles, vapors, and gases. The energy for mobile sources such as automobiles, trucks, and airplanes is generally provided by petroleum products that upon combustion emit many organic chemicals into the air. Most manufacturing processes, especially in the chemical industries, create and discharge some contaminants into the environment. In addition, chemicals that provide certain benefits to society, such as some pesticides, food preservatives, and cancer chemotherapy agents, have been shown to have genotoxic effects.

For many years, researchers and public health specialists have contemplated what effect these agents may have on public health. Since most exposures to many of these products are chronic, low-level exposures, the greatest concern has been over mutagenicity, carcinogenicity, and teratogenicity.

Identifying genotoxic hazards can be accomplished by epidemiological studies, whole animal studies, short-term tests, or a combination of the three. Epidemiology is potentially the most definitive; however, this approach is generally difficult, costly, and must be made after humans are exposed. Whole animal studies are usually the next best approach, but in addition to

being slow and expensive, they require relatively large amounts of the substance in question and suffer from extrapolation problems. *In vitro* tests for genotoxicity are more rapid and less expensive, but also suffer from the need to correlate any results with potential animal or human effects. The combining of *in vitro* tests with whole animal and human exposure situations, can provide important exposure assessment information but does not necessarily overcome the extrapolation problems between the different genotoxicity end points.

Since interaction with deoxyribonucleic acid (DNA) defines the genotoxic end points detected, simple *in vitro* tests can be used to monitor or screen most chemicals or mixtures of chemicals for specific genotoxic activity. Bacterial tests to screen classes of chemicals for gene mutations can provide a screen for many potential mutagens, carcinogens, and teratogens. Since the biochemical mechanisms for all mutagens, carcinogens, and teratogens are not the same, however, bacterial tests cannot be expected to detect all genotoxicants. Only by clearly understanding a test's usages and limits, can one most effectively use the information provided by that test. An overview of bacterial mutagenicity testing follows.

Mutagenicity tests using bacteria have been available for approximately 30 years. In 1951, Demarec et al. used an *Escherichia coli* (*E. coli*) reverse mutation system to test 31 chemicals. Szybalski (1958) tested 431 substances for mutagenicity using a somewhat different *E. coli* system. In 1971, Drs. Ames and Yamasaki published a mutagen detection system using histidine and requiring mutants of *Salmonella typhimurium* (*S. typhimurium*). As investigators began to use this test, many procedural modifications were made to improve the original protocol. One of the most significant improvements to *in vitro* tests was shown by Malling (1971) who used a mammalian metabolizing system to activate dimethylnitrosamine to its mutagenic metabolite. Ames also developed more sensitive *S. typhimurium* test strains. Combining these improvements, Ames et al. (1975) published a detailed protocol that incorporated the use of a mammalian metabolizing system, and established the Ames test as a reliable screening system for mutagenicity and potential carcinogenicity. Many have examined, discussed, and suggested modifications and additions to the Ames 1975 protocol (Arimoto et al., 1981; Grafe et al., 1975; Belser et al., 1981; Salmeen and Durstin, 1981; Griem, 1980; Polley and Raineri, 1980; de Serres and Shelby, 1979; Cheli et al., 1980; Thompson and Melampy, 1981; Chen et al., 1975; Shahin and von Borstel, 1978; Matsumoto et al., 1977; Anderson and McGregor, 1980; Booth et al., 1980; and Dunkel, 1979). No other protocol has gained as much general and standard

acceptance. A discussion of the Ames test procedure and its special uses, limitations, and controversial aspects is the aim of this chapter.

BASIC THEORY AND PROTOCOL

The procedure, developed by Ames et al. (1975), detects reverse mutations that occur in histidine-requiring strains of S. typhimurium. The original indicator strains selected were either spontaneous or induced mutants of the parental strain LT-2, that will not grow on histidine-free medium. In the Ames test when these histidine-requiring mutant strains undergo a reverse mutation to prototrophy (wild type), the mutants (often referred to as revertants) can be detected on a minimal nutrient medium that either lacks or is deficient in histidine. Since more than one type of gene mutation can be induced in bacterial DNA, more than one indicator strain had to be selected. Two broad mutational classes of indicator strains exist: one class consists of those strains that must undergo a base-pair substitution to revert to prototrophy; the other class consists of those strains that respond to frameshift mutagens.

Besides the histidine-locus mutation, the most commonly used strains also have additional mutations incorporated. First, the rfa mutation creates a lipopolysaccharide deficiency in the bacterial cell wall, increasing the cell's permeability to large molecules. The uvr B mutation was incorporated into the frequently used strains to increase their susceptibility to several classes of mutagens by impairing genetic repair. In addition, the PKM101 R-factor plasmid, a modification found in some strains, appears to increase the indicator strain's sensitivity by participating in a type of error-prone repair. Nitroreductase deficiency has been incorporated in some strains to prevent bacterial metabolism of nitroarenes. The five strains routinely used by most mutagenicity laboratories, TA98, TA100, TA1535, TA1537, and TA1538, are listed in Table 1, together with some of the less commonly used strains.

The Ames test usually refers to the plate incorporation technique, outlined in Figure 1. With this type of protocol, the bacteria, the substance to be tested, and any metabolic activating system used are mixed in a melted, soft-agar overlay and then poured onto minimal media plate. The medium basically consists of a salts solution, glucose, agar, biotin, a trace amount of histidine, and water. The added trace of histidine allows the bacteria to replicate while exposed to the substance being tested, and causes a cloudy background growth ("lawn") of bacteria upon

Table 1. Specific Mutations in *Salmonella typhimurium* Strains Used for Mutagenicity Testing

Strain	Specific Mutations			
	rfa	Δ *uvr* B	R-Factor	Nitroreductase Deficiency
TA98	P[a]	P	P	A[b]
TA100	P	P	P	A
TA1535	P	P	A	A
TA1537	P	P	A	A
TA1538	P	P	A	A
TA1978	P	A	A	A
TA98/NR	P	P	P	P[c]
TA98/1,8 DNP_6	P	P	P	P[d]
G46	A	A	A	A

[a]P = Presence.
[b]A = Absence.
[c]Deficient in more classical nitroreductase enzyme.
[d]Deficient in enzyme system that metabolizes 1,8 dinitropyrene.

the plate, but the concentration is not enough to produce individual colonies.

Using an agar medium places limits on the test. First, because not all substances are soluble in this medium, the test substance may not be made available to the bacteria. Second, both the variety and amount of solvent material used are limited by the nature of an agar medium, as well as by the toxicity of the solvent to the bacteria. Third, some compounds volatilize out of the medium very rapidly. Fourth, although preparing the medium seems simple, small changes in preparation can have major effects. For example, if the biotin solution is added when the medium is too hot, the biotin may be inactivated and the number of bacteria surviving on the plate decreased. But if the medium is too cool when the biotin is added, uneven mixing of the solutions may occur and bacterial survival will differ among plates. Cautious care of the agar medium cannot be overemphasized.

In regard to solvents, dimethyl sulfoxide (DMSO) is the most commonly used, although a variety of other solvents including

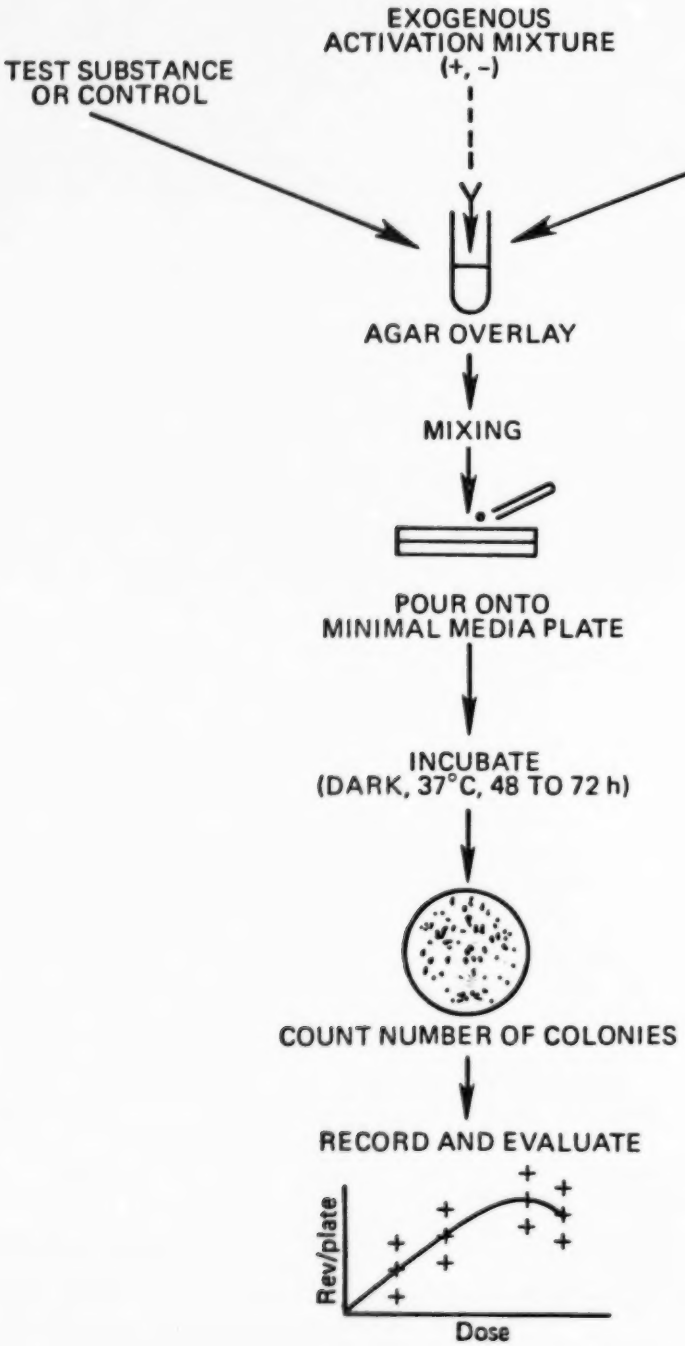

Figure 1. Outline of the Ames bioassay testing procedure.

water, acetone, and ethanol can be used. Most solvents, however, are somewhat toxic to bacteria.

Compounds that produce a mutagenic response in the bacterial test without adding a mammalian metabolic activating system are generally called direct-acting mutagens. This terminology is often used even though bacterial metabolism may have to occur before a compound is mutagenic within the test system. Some compounds may require that a mammalian enzyme system be present before detecting mutagenicity in the bacterial test, and these compounds are usually called indirect mutagens or promutagens. Therefore, mutagens that are identified by in vitro tests fall into three categories: (1) the direct-acting (also called ultimate) mutagens, (2) those requiring endogenous activation (metabolism exhibited by the indicator organism), and (3) those requiring exogenous metabolism (metabolism not provided by the indicator organism). Also, note that endogenous and exogenous activation can either activate or deactivate a compound.

Exogenous metabolism is usually provided by a tissue homogenate that is added to the agar overlay before pouring it onto the plate. The most commonly used homogenate is an Aroclor-induced rat liver 9000 x g homogenate referred to as S9. The Aroclor induces the mixed function oxygenase (MFO) system of enzymes, thus promoting increased metabolite levels, and producing corresponding increases in revertant colonies when these metabolites are mutagenic. Because S9 contains microsomal plus cytosol enzymes, this simple, yet effective method provides exogenous metabolic activation. Among the many variations that can be introduced in the exogenous activation portion of the assay are changes in strain or species of animal, type of induction, type of activation fraction (e.g. microsomes, whole cells), and the methods of both application and exposure.

To conduct the Ames assay, Salmonella broth, a sample, and some S9 mix are added to a melted overlay in a small test tube. The mixture is gently vortexed, poured onto a minimal media plate as a smooth even layer, and allowed to harden. These plates are then inverted and incubated in darkness at 37°C for 48 to 72 hours. This process is repeated for each replicate of each dose tested and for each control. If testing a single compound with five tester strains both with and without S9, and there are three replicates at five doses, 150 total plates would be used for the test substance. In addition, 50 to 60 plates would be needed for spontaneous, positive, and sterility controls.

After incubation, plates are examined for background growth, contamination, and number of colonies. The background growth or lawn, due to the trace histidine added to the media, causes the plates to look cloudy when held up toward a light source. Clearing of this background indicates that the sample or solvent are toxic to the bacteria. An overgrowth indicates the presence of histidine or histidine-like substitutes. Colonies should be white, evenly growing (though rough edged), and of moderate size. Some colonies grow on the surface rather than within the overlay, and will be slightly larger. Colonies can also exhibit the color of any highly colored agent tested. All other colony types should be considered as contaminants and noted as such.

After the plates are examined, the number of colonies per plate are counted and recorded. Normally counts range from zero to 2000 although greater colony counts have been reported. While most electronic counters cannot count accurately above a range of 1500 to 1800, the reliability of hand counting decreases rapidly for counts exceeding 500. All data counts and additional information must be carefully recorded. Data summary and evaluation will be discussed in another section.

While the suggested minimum incubation time is 48 hours, our recent work indicates that results are more consistent with 72-hour incubation. This may be due to the toxicity of a compound or complex mixture inhibiting the growth of any revertant colonies. However, proper adjustment of the histidine level is critical if incubating plates for 72 hours. Another change from the Ames et al. 1975 protocol is the use of Oxoid #2 broth instead of Difco Nutrient Broth for the growth of overnight cultures. The Oxoid #2 broth consistently provides a higher density of viable cells.

Correct microbiological techniques are needed at every step of the assay. For example, rigorous attention must be paid to sterility, especially that of the sample, S9, and media. Contaminating bacterial colonies may not be easily recognized and therefore could alter the revertant count. Tests should be performed in biological safety hoods that provide a sterile environment for the test and protection for the worker. Work done under yellow light prevents photodeactivation of any test substance. Also, the best quality incubators available should be used since more than a 1°C change can alter the results.

SPECIALIZED PROTOCOLS

While the plate incorporation test protocol is sufficient to screen most substances, taking full advantage of rapid microbial techniques depends upon understanding the variety of alternative procedures available. This section provides brief overview of these alternative procedures.

Spot Test

The spot test is a qualitative version of the plate incorporation test. Differing concentrations of the sample are placed in the center of minimal media plates (previously prepared with bacteria and any activating system). After proper incubation, plates are visually inspected for circular zones of killing and increased mutant colonies around the points of application. This technique is useful when surveying many samples in a short time period. However, spot testing has two primary drawbacks. First, interpretation may be difficult since the zones of killing and mutagenicity can be very narrow. Second, the test relies upon the substance under test to produce a gradient throughout the medium, but many substances do not readily diffuse through an aqueous media (Ames, 1971).

Fluctuation Test

In this assay the tester strains, the test substance, and an acid-base indicator are mixed in a liquid medium and dispensed into approximately 50 tubes or wells. The test is scored by counting the number of tubes or wells that change color. If a mutation arises, a mutant clone develops. As the mutant clone grows, sugar utilization produces acid, altering the color of the acid-base indicator. A positive response is indicated by a change of color, indicating mutant cell growth, in a significant number of tubes (Green et al., 1976, 1977).

Preincubation Test

In this test the compound is incubated in the overlay tube with only the bacteria and activation mixture for a predetermined length of time. The overlay agar is added to the tube after incubation and the mixture is then poured as usual onto a minimal media plate. Several advantages occur with this technique. First, some compounds, notably nitrosamines, are negative in the normal plate test but are positive with this protocol. Second, a portion of the preincubation mixture can be tested on a complete media plate, enabling an index of survival to be calculated.

Third, this test can be done in sealed vials when testing volatile chemicals. However, one drawback is the significant increase in labor involved.

The Suspension Test

The principles, advantages, and disadvantages of this method are similar to those of the preincubation test. In this test however, larger volumes are used in stoppered flasks, flasks are incubated in a rotary bath, and multiple plates (for both mutation and survival counts) can be poured from each flask. This technique allows more precision and replication at each dose, as well as better mixing especially for volatile compounds. However, it is even more time consuming than the preincubation test, and therefore seldom used today (Frantz and Malling, 1975).

Gas and Vapor Testing

Testing gases and vapors can be either very complex or quite simple. The type of testing done depends upon the available materials and equipment, the precision needed in the final data, the nature of the compound, and the questions to be answered (Barber et al., 1981; McCoy et al., 1978; Bartsch et al., 1975; Rannug et al., 1978; Baden et al., 1978).

Glass desiccators. Most testing of gases has been done using glass desiccators. Plates spread with bacteria (and any activating system used) are opened inside a desiccator and the desiccator sealed. The gas or vapor is then injected into the chamber and the exposed plates are allowed to incubate.

Inverted plate techniques. Here, plates are first spread with the bacteria and activating system and then inverted. While inverted, the liquid form of the compound is placed on the lid of the petri dish (which is below the bacteria and medium), and then sealed inside a plastic bag. The bacteria are exposed as the compound volatilizes.

Specialized chambers. Recognizing the inadequacies of the previously mentioned techniques investigators have designed several exposure chambers that are being tested. These chambers have been designed to overcome such problems as the bulkiness and weight of glass dessicators, uneven mixing, the interaction of the substance with chamber walls, and the uptake of the gas and vapor by the test organism. This work is too diverse for a complete overview here.

Body Fluid Testing

One major *in vitro* test advance was the incorporation of a mammalian metabolic activating system. However, *in vitro* preparations of tissue homogenates do not duplicate complete *in vivo* metabolism. For example, safrole is a procarcinogen but is not metabolized to a mutagen by *in vitro* preparations of S9 liver homogenate (Rosenkranz and Poirier, 1979). The testing of body fluids from exposed and unexposed animals or humans is one method of examining the mutagenicity of compounds exposed to whole-animal metabolism. In addition, it can be used to explore how factors such as diet, enzyme induction, and tissue exposure affect mutagenicity.

One particularly rewarding use of body fluid analysis is in human epidemiology studies. In 1977, Yamasaki and Ames demonstrated that urine collected from smokers was mutagenic. A more definitive study on urine mutagenicity from cigarette smokers was completed by Van Doorn et al., 1979. Investigators have also detected mutagenic activity in the urine of patients under drug treatment and drugs giving positive mutagenic response include metronidazole, niridazole (Legator et al., 1975) and cyclophosphamide (Minnick et al., 1976). Mutagenic urines have also been detected from certain types of occupational exposures, and includes nurses handling cytostatic drugs (Falck et al., 1979), and Finnish rubber workers (Falck et al., 1980). However, investigators must understand that effects in humans are not being measured in these studies, and that only exposure to a genotoxic agent is being confirmed.

Other Procedural Modifications

A variety of other types of protocols and specialized modifications have been used by many investigators. The host-mediated assay (Gabridge and Legator, 1969) allows both the injection of an indicator cell line into an exposed host animal, and the recovery of the indicator cell line for mutagenicity assay. The well test (Hughes et al., 1978) is a modification of the spot test that allows multiple conditions to be assayed with a single plate.

TESTING CRITERIA

Individual substances vary not only in genotoxicity, but also in solubility, mode of action, and toxicity to the indicator organism. All of these factors must be considered when planning mutagenesis experiments. Initial testing should give the

investigator definite information about toxicity, solubility, and any inherent technical problems with the substance being tested. Many investigators do a toxicity range-finding assay before performing the mutagenicity assay. This author prefers to perform a preliminary mutagenicity test so that information about potential mutagenicity can be incorporated into the design of the more definitive tests. Helpful guidelines include:

1. Minimum compound testing is at 5 doses spaced at half-log intervals;

2. Agents that either precipitate or demonstrate an apparent loss of the compound due to volatility are retested under modified conditions;

3. Compounds that demonstrate little or no toxicity are tested in the 5 to 10 mg range before being reported as negative;

4. When possible, triplicate plates per dose are used and the whole test is repeated at least once;

5. If a test appears positive, colonies are selected from an induced-colony plate and plated on minimal media to determine whether they are true mutants.

SUMMARIZING AND ANALYZING DATA

For several years, there has been a general consensus rule for judging the mutagenicity of a substance as either negative or positive in this assay. The rule states that a substance producing a regularly increasing dose-response curve and that at one or more doses the revertants per plate were greater than double the spontaneous count was positive. All other results were to be regarded negative. This rule is adequate for most substances, since most bacterial mutagens give dramatic responses in one or more tester strains. However, some substances, give repeatable dose-responsive results without a doubling of the spontaneous count. This occurs when toxicity masks mutagenicity, or when the compound precipitates, but interpretating the results for such compounds could not be agreed upon. This controversy sparked the use of several simple types of analysis (Chu et al., 1981; Weinstein and Lewinson, 1978) that however, were not always acceptable. In the last three years several statistical modeling methods for the Ames assay have ben proposed (Stead et al., 1981; Myers et al., 1981; and Margolin et al., 1981) and each approach has definite strengths and weaknesses. While the most sound and

applicable approach has not yet been determined, we can explore and compare these methods. In the interim, most data analysis can be effectively accomplished by using the consensus rule and by calculating the slope of the linear portion of the dose-response curve. However, since dose-response curves are not truly linear, a minimum of four doses (which includes the zero control dose) should be used in calculating this linear slope.

SUMMARY

The S. typhimurium mammalian activation tests for mutagenicity, commonly referred to as the Ames assay, is a rapid, reliable bacterial test for mutagenicity. It is a plate incorporation assay that uses selected histidine-deficient strains derived from S. typhimurium LT-2. Upon treatment with certain types of mutagens the mutant strains revert back to prototrophy, produce histidine, and grow on selective media. The test can be completed in four to five days at minimal costs and can be modified in several ways to explore various aspects of mutation testing. The assay can be used to screen individual compounds, and simple or complex mixtures. Many substances have been tested with the assay, providing one of the largest data bases in genetic toxicology testing. Despite increasing debate over the best methods for the data analysis, the qualitative interpretation of results is usually easy and reliable. Ample utility of the test has been demonstrated and the use of bacterial mutagenicity for screening mutagens and potential carcinogens will continue as a broad and useful field of toxicology.

REFERENCES

Ames, B. N., and Yamasaki, E., 1971, The detection of chemical mutagens with enteric bacteria (Chapter 9) in: "Chemical Mutagens: Principles and Methods for their Detection, Vol. I," A. Hollander, ed., Plenum Press, New York.

Ames, B. N., McCann, J., and Yamasaki, E., 1975, Methods for detecting carcinogens and mutagens with the Salmonella/ mammalian - microsome mutagenicity test, Mutat. Res., 31:347-364.

Anderson, D., and McGregor, D. B., 1980, The effect of solvents upon the yield of revertants in the Salmonella/activation mutagenicity assay, Carcinogenesis, 1:363-366.

Arimoto, S., Negishi, K., and Hayatsu, H., 1981, A modification of the Ames test procedure: accelerated growth of the his^+revertants, Mutat. Res., 91:407-411.

Baden, J. M., Kelley, M. S., Simmons, V. F., Rice, S. A., and Mazze, R. I., 1978, Fluroxene mutagenicity, Mutat. Res., 58:183-191.

Barber, E., Donish, W., and Mueller, K., 1981, A procedure for the quantative measurement of the mutagenicity of volatile liquids in the Ames Salmonella microsome assay, Mutat. Res., 90:31-48.

Bartsch, H., Malaveille, C., and Montesano, R., 1975, Human, rat and mouse host-mediated mutagenicity of vinyl chloride in S. typhimurium strains, Int. J. Cancer, 15:429-437.

Belser, W. L., Shaffer, S. D., and Bliss, R. D., 1981, A standardized procedure for quantification of the Ames Salmonella/mammalian-microsome mutagenicity test, Environ. Mutagenesis, 3:123-139.

Booth, S., Welch, A., and Colin, R., 1980, Some factors affecting mutant numbers in the Salmonella/microsome assay, Carcinogenesis, 1:911-923.

Bridges, B. A., 1978, On the detection of volatile liquid mutagens with bacteria: experiments with dichlorovos and epichlorohydrin, Mutat. Res., 54:367-371.

Cheli, C., Defrancesco, D., Petrullo, L. A., McCoy, E. C. and Rosenkranz, H. S., 1980, The Salmonella/mutagenicity assay: reproducibility, Mutat. Res. 74:145-150.

Chen, C. C., Speck, W. T., and Rosenkranz, H. S., 1975, Mutagenicity testing with Salmonella typhimurium strains, II, the effect of unusual phenotypes on the mutagenic response, Mutat. Res., 28:31-35.

Chu, K., Patel, K., Lin, A., Tarone, R., and Linhart, M., and Dunkel, V., 1981, Evaluating statistical analyses and reproducibility of microbial mutagenicity assays, Mutat. Res., 85:119-132.

Demarec, M., Bertani, G., and Flint, J., 1951, A survey of chemicals for mutagenic action of E. coli, Am. Naturalist, 85:119-136.

de Serres, F. J., and Shelby, M. D., 1979, Recommendations on data production and analysis using the Salmonella/microsome mutagenicity assay, Environ. Mutagenesis, 1:87-92.

Dunkel, V. C., 1979, Collaborative studies on the Salmonella/ microsome mutagenicity assay, J. Assoc. Off. Anal. Chem., 62(4):874-882.

Falck, K., Grohm P., Sorsa, M., Vainio, H., Heinonen, E., and Holsti, L., 1979, Mutagenicity of urine in nurses handling cytostatic drugs, Lancet, 1979:1250-1251.

Falck, K., Sorsa, M., and Vainio, H., 1980, Mutagenicity in urine of workers in rubber industry, Mutat. Res., 79:45-52.

Frantz, C. N., and Malling, H. V., 1975, The quantitative microsomal mutagenesis assay method, Mutat. Res., 31:365-380.

Gabridge, M. G., and Legator, M. S., 1969, A host-mediated microbial assay for the detection of mutagenic compounds, Proc. Soc. Exp. Biol. Med., 130:831-834.

Grafe, A., Lorenz, R., and Vollmar, J., 1975, Testing the mutagenic potency of chemical substances in a linear host mediated assay, Mutat. Res., 31:205-216.

Green, M. H. L., Bridges, E. A., Rogers, A. M., Horspool, G., Muriel, W. J., Bridges, J. S., and Fry, J. R., 1977, Mutagen screening by a simplified bacterial fluctuation test: use of microsomal preparations and whole liver cells for metabolic activation, Mutat. Res., 48:287-294.

Green, M. H. L., Muriel, W. J., and Bridges, B. A., 1976, Use of a simplified fluctuation test to detect low levels of mutagens, Mutat. Res., 38:33-42.

Greim, H., 1980, Mutagenicity testing with Salmonella microsome test, Arch. Toxicol., 46:31-40.

Hughes, T. J., Little, L., Claxton, L., Waters, M., Pellizzarri, E., and Sparacino, C., 1978, Application of agar diffusion wells for microbial mutagenicity testing of air pollutants, Poster Seminar Abstract for Ninth Annual Meeting of the Environmental Mutagen Society, Nashville, Tenn.

Legator, M. S., Connor, T., and Stoeckel, M., 1975, Detection of mutagenic activity of metronidazole and niridazole in body fluids of humans and mice, Science, 188:1118-1119.

Malling, H. V., 1971, Dimethylnitrosamine: formation of mutagenic compounds by interaction of mouse liver microsomes, Mutat. Res., 13:425-429.

Margolin, B. H., Kaplan, N., and Zeiger, E., 1981, Statistical analysis of the Ames Salmonella/microsome test, Proc. Natl. Acad. Sci., USA, 78(6):3779-3783.

Matsumoto, T., Yoshida, D., and Mizusaki, S., 1977, Enhancing effect of harman on mutagenicity in Salmonella, Mutat. Res., 56:85-88.

McCoy, E., Burrows, L., and Rosenkranz, H. S., 1978, Genetic activity of allyl chloride, Mutat. Res., 57:11-15.

Minnick, V., Smith, M. E., Thompson, D. T., and Kornfeld, S., 1976, Detection of mutagenic activity in human urine using mutant strains of Salmonella typhimurium, Cancer, 38:1253-1258.

Myers, L. E., Sexton, N. H., Southerland, L. I., and Wolff, T. J., 1981, Regression analysis of Ames test data, Environ. Mutagenesis, 3:575-586.

Polley, J., and Raineri, R., 1980, Metabolic activation by hamster and rat hepatocytes in the Salmonella mutagenicity assay, J. Natl. Cancer Inst., 65:1293-1298.

Rannug, U., Sandvall, A., and Ramel, C., 1978, The mutagenic effect of 1,2-dichloroethane on Salmonella typhimurium, I, activation through conjugation with glutathione in vitro, Chem.-Biol. Interact., 20:1-16.

Rosenkranz, H. S., and Poirier, L. A., 1979, Evaluation of the mutagnicity and DNA modifying activity of carcinogens and noncarcinogens in microbial systems, J. Natl. Cancer Inst., 873-891.

Salmeen, I., and Durstin, A., 1981, Some effects of bacteria population on quantitation of Ames Salmonella histidine reversion mutagenesis assays, Mutat. Res., 85:109-118.

Shahin, M. M. and von Borstel, R. C., 1978, Comparisons of mutation induction in reversion systems of Saccharomyces cerevisiae and Salmonella typhimurium, Mutat. Res., 53:1-10.

Stead, A. G., Hasselblad, V., Creason, J. P. and Claxton, L., 1981, Modeling the Ames test, Mutat. Res., 85:13-27.

Szybalski, W., 1958, Special microbiological systems, II, observations on chemical mutagenesis in microorganisms, Ann. NY Acad. Sci. 76:475-489.

Thompson, E., and Melampy, P., 1981, An examination of the quantitative suspension assay for mutagenesis with strains of Salmonella typhimurium, Environ. Mutagenesis, 3:453-465.

Van Doorn, R. B., Wagenaas-Zegers, M., Leijdekkers, M., Therews, J., and Henderson, P., 1979, Thioether concentration and mutagenicity of urine from cigarette smokers, Int. Arch. Occupat. Environ. Health, 43:159-166.

Weinstein, D., and Lewinson, T. M., 1978, A statistical treatment of the Ames mutagenicity assay, Mutat. Res., 51:433-434.

Yamasaki, E., and Ames, B. N., 1977, Concentration of mutagens from urine by adsorption with the nonpolar resin XAD-2: cigarette smokers have mutagenic urine, Proc. Natl. Acad. Sci., USA, 74(8):3555-3559.